UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Note to Reader

Background: As part of its effort to involve the public in the implementation of the Food Quality Protection Act of 1996 (FQPA), which is designed to ensure that the United States continues to have the safest and most abundant food supply. EPA is undertaking an effort to open public dockets on the organophosphate pesticides. These dockets will make available to all interested parties documents that were developed as part of the U.S. Environmental Protection Agency's process for making reregistration eligibility decisions and tolerance reassessments consistent with FQPA. The dockets include preliminary health assessments and, where available, ecological risk assessments conducted by EPA, rebuttals or corrections to the risk assessments submitted by chemical registrants, and the Agency's response to the registrants' submissions.

The analyses contained in this docket are preliminary in nature and represent the information available to EPA at the time they were prepared. Additional information may have been submitted to EPA which has not yet been incorporated into these analyses, and registrants or others may be developing relevant information. It's common and appropriate that new information and analyses will be used to revise and refine the evaluations contained in these dockets to make them more comprehensive and realistic. The Agency cautions against premature conclusions based on these preliminary assessments and against any use of information contained in these documents out of their full context. Throughout this process, If unacceptable risks are identified, EPA will act to reduce or eliminate the risks.

There is a 60 day comment period in which the public and all interested parties are invited to submit comments on the information in this docket. Comments should directly relate to this organophosphate and to the information and issues available in the information docket. Once the comment period closes, EPA will review all comments and revise the risk assessments, as necessary.

These preliminary risk assessments represent an early stage in the process by which EPA is evaluating the regulatory requirements applicable to existing pesticides. Through this opportunity for notice and comment, the Agency hopes to advance the openness and scientific soundness underpinning its decisions. This process is designed to assure that America continues to enjoy the safest and most abundant food supply. Through implementation of EPA's tolerance reassessment program under the Food Quality Protection Act, the food supply will become even safer. Leading health experts recommend that all people eat a wide variety of foods, including at least five servings of fruits and vegetables a day.

Note: This sheet is provided to help the reader understand how refined and developed the pesticide file is as of the date prepared, what if any changes have occurred recently, and what new information, if any, is expected to be included in the analysis before decisions are made. **It is not meant to be a summary of all current information regarding the chemical.** Rather, the sheet provides some context to better understand the substantive material in the docket (RED chapters, registrant rebuttals, Agency responses to rebuttals, etc.) for this pesticide.

Further, in some cases, differences may be noted between the RED chapters and the Agency's comprehensive reports on the hazard identification information and safety factors for all organophosphates. In these cases, information in the comprehensive reports is the most current and will, barring the submission of more data that the Agency finds useful, be used in the risk assessments.

Jack E. Housenger, Acting Director
Special Review and Reregistration Division

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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WASHINGTON, D.C. 20460

HED DOC. NO. 013710

August 12, 1999

MEMORANDUM

SUBJECT: **PHOSALONE.** Report of the Hazard Identification Assessment Review Committee.

From: Kit Farwell, D.V.M.
Reregistration Branch I
Health Effects Division 7509C

Through: Pauline Wagner, Co-Chair
and
Jess Rowland, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

To: Whang Phang, Ph.D., Branch Senior Scientist
Reregistration Branch I
Health Effect Division 7509C

PC Code 097701

On July 22, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of **PHOSALONE** and established acute and chronic Reference Doses (RfDs) for an import tolerance. The HIARC also addressed the potential enhanced sensitivity of infants and children from exposure to phosalone as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Committee Members present: William Burnam, Susan Makris, David Anderson, Jess Rowland, Nicole Paquette, Virginia Dobozy, P.V. Shah, Pam Hurley, Nancy McCarrol, Tina Levine, and Kathleen Raffaele.

Executive Secretary: Brenda Tarplee

Members in absentia: Karen Hamernik, Mike Ioannou, Pauline Wagner.

Data was presented by Dr. Kit Farwell of Reregistration Branch 1.

In attendance, also: William Hazel, risk assessor for phosalone.

Data Presentation and Report Presentation:	Kit Farwell Toxicologist

I. INTRODUCTION

Phosalone is an organophosphate pesticide. All U.S. uses were withdrawn in 1989. Use on the following crops is being supported for import purposes only: almond, apples, apricots, cherries, grapes, peaches, pears, and plums/prunes. It is assumed that uses will be late season resulting in significant residues on fruits/nuts; current tolerances are 10 or 15 ppm on the above crops (40 CFR 180.263). Physical properties include the following:

Physical state.	Solid
Melting point.	40 C
Solubility.	Water: 0.017 g/L, Hexane: 11.65 g/L, Methanol: 237 g/L
Vapor pressure.	0.96 x 10-6 mmHg at 40 C (<0.5 x 10^{-6} mmHg at 24 C)
Octanol/H_2O coeff.	5.89 x 10^3
Stability.	Fairly stable to heat and at pH 5 & 7; moderately at pH 9

Acute and chronic dietary endpoints were selected. Dermal and inhalation endpoints for occupational and residential exposure were not selected since the action is for an import tolerance. The HIARC addressed the potential enhanced sensitivity of infants and children as required by the Food Quality Protection Act (FQPA) of 1996.

II. HAZARD IDENTIFICATION

A.1. Acute RfD Subpopulation: Females 13+ years of age

Study Selected: Developmental Toxicity in Rabbits OPPTS 870.3700 (OPP §83-3a)

MRID No.: 41089501

Executive Summary: In a developmental toxicity study (MRID 41089501) 4 groups of pregnant Chinchilla Kfm: CHIN, Hybrids, SPF Qualified rabbits (16/dose) were given phosalone technical (93.5% a.i.) in 4% CMC (by gavage) at dose levels of 0, 1, 10, and 20 mg/kg/day from days 6 through 18 of gestation. The test article was administered at a volume 4 ml/kg body weight. Observations for maternal toxicity or mortality were conducted twice a day from GD 0 - 28. On GD 28, the dams were euthanized and their uteri and ovaries were examined. The fetuses were examined both viscerally and externally. Cholinesterase activity was **not** measured in this study.

A statistically significant decrease ($p \leq 0.05$) in food consumption, during the dosing period only, was observed in high-dose (20 mg/kg/day) group animals. A slight but not statistically significant overall weight loss and a decrease in body weight gain were reported for animals in this dose group. Animals in the high-dose group also exhibited evidence of dyspnea (12/16 animals), abdominal cramps (8/16 animals), and extension spasms and/or convulsions and/or lying prostrate (5/16 animals).

Under the conditions of this study, the **maternal LOAEL** is established at **20 mg/kg/day** (HDT) based on signs of toxicity (dyspnea, abdominal cramps, convulsions, etc.) and a statistically significant decrease in food consumption. The **maternal NOAEL** is **10 mg/kg/day**.

Developmental Toxicity - While the pregnancy rates were not affected by treatment with the test article, the statistically significant increased incidence of post-implantation losses reported at the 10 and 20 mg/kg/day dose levels ($p \leq 0.05$ and $p \leq 0.01$, respectively) suggest a compound-related effect. Total embryonic resorption was also noted for animals in these groups (2/16 at the high-dose and 1/16 at the mid-dose) while none was seen in the control. Mean fetal body weight did not appear to be affected by treatment with the test article. Under the conditions of this study, the **developmental LOAEL** is established at **10 mg/kg/day** based on post-implantation losses. The **developmental NOAEL** is established at **1 mg/kg/day**.

This study in the rabbit is **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3) in rabbits.

Dose Selected for Risk Assessment: Developmental NOAEL = 1 mg/kg/day based upon post-implantation losses at the LOAEL of 10 mg/kg/day.

Uncertainty Factors: 100x (10x for interspecies extrapolation and 10x for intraspecies variabililty).

$$\textbf{aRfD (Females 13+)} = \frac{\text{1.0 mg/kg/day (NOAEL)}}{\text{100 (UF)}} = \textbf{0.01 mg/kg/day}$$

Comments about Study/Endpoint: An increase in fetal resorptions is an effect which could occur after a single exposure. The selected endpoint is a very conservative indicator of toxicity because it is based on total resorptions and is not a litter effect. Mean resorptions were increased but not with statistical significance. Total resorptions achieved statistical significance but were primarily attributed to 1 dam with total resorptions in the mid-dose group and 2 dams with total resorptions in the high-dose group. **This risk assessment is required and applies only to females of 13 years age or greater.**

A.2. Acute RfD Subpopulation: General Population (including infants and children)

Study Selected: Acute Neurotoxicity in Rats. (OPPTS 870.6200; §81-8)

MRID No.: 44852503

Executive Summary: In an acute oral neurotoxicity study (MRID 44852503) 4 groups of 28 - 35 days old Crl: CD BR rats were given a single oral dose (by gavage) of Phosalone Technical (93.8% a.i.), in corn oil at doses of 0, 10, 25, or 60 mg/kg of body weight. The study consisted of two sets of experiments: 1) the main study groups contained 10 rats/sex/dose and were used for neurobehavioral testing and clinical signs observations; 2) the satellite group contained 5 rats/sex/dose and were used for determination of cholinesterase activity. All animals were observed daily for signs of toxicity and twice a day for signs of moribundity and/or mortality.

Clinical signs such as brown nasal staining, damp urogenital area, loose feces, and wet lower jaw fur were first reported at the 25 mg/kg dose level approximately 3 - 4 hours after exposure to the test article (3/10 females and 1/10 males in the main group; and 4/8 females and 3/5 males in the satellite group).

At the 60 mg/kg dose level, 20/20 females (10 main group, 10 satellite group) and 12/15 males (7/10 main group, 5/5 satellite group) showed a myriad of clinical signs including tremors, hunched posture, clonic jaws, unsteady gait, cold extremities, exophthalmia, and wet/damp urogenital/anogenital region approximately 3 - 4 hours after exposure to the test substance. With the exception of fur staining, all clinical signs of toxicity had resolved by the 24 hour examination period. No mortalities were reported at any dose level during the study period. A slight but statistically significant decrease in body weight gain was noted for males at the 60 mg/kg dose level during the first week of the study. A concomitant decrease in food consumption (not statistically significant) was also noted for this time period.

The first indications of compound-related effects detected by the Functional Operational Battery (FOB) were reported in both males and females at the 60 mg/kg dose during the 6 hour post-dosing observation period and included tremors, exophthalmia, clonic jaws, piloerection, unusual gait (walking on toes), hypothermia, decreased activity, and wet anogenital region. These symptoms resolved within the first week after treatment with the test article and were no longer evident at the Day 8 observation period.

A statistically significant inhibition of plasma cholinesterase activity (PChE) was reported at all dose levels six hours after exposure to the test article (time of peak effect). This inhibition persisted at the 60 and 25 mg/kg (males only) dose levels for 24 hours but was no longer evident at the Day 7 observation period. Additionally, a statistically significant RBC ChE inhibition, which persisted during the first 24 hours of the study period, was noted at the 60 mg/kg dose level. Animals in the high-dose group (60 mg/kg) showed evidence of statistically significant plasma ChE inhibition at the Day 15 examination (end of the study period).

No compound-related abnormalities were found in histological examination. Under the conditions of this study, the **LOAEL** is established at **10 mg/kg** based on plasma cholinesterase inhibition in males and females. No clear **NOAEL** could be established for this study. This study is classified **acceptable/guideline** and satisfies the guideline requirement for an acute neurotoxicity study (OPPTS 870.6200; §81-8) in rats.

Dose Selected for Risk Assessment: LOAEL = 10 mg/kg/day based on plasma cholinesterase inhibition.

Uncertainty Factors: 300x (10x for interspecies extrapolation, 10x for intraspecies variabililty, and 3x for lack of a NOAEL).

$$\textbf{aRfD (general population)} = \frac{\text{10 mg/kg/day (LOAEL)}}{\text{300 (UF)}} = \textbf{0.03 mg/kg/day}$$

Comments about Study/Endpoint: The duration of exposure in this study is appropriate for an acute dietary risk assessment. Plasma ChE inhibition is a sensitive endpoint for phosalone. Although a NOAEL for plasma cholinesterase was not determined in this study, the LOAEL is believed to be close to a NOAEL, as neither brain nor RBC cholinesterase were statistically significantly inhibited at 10 mg/kg or 25 mg/kg in this study. **This risk assessment is required.**

B. Chronic RfD

Study Selected: Combined Chronic/Carcinogenicity Study in Rats. 870.4300 (§83-5)

MRID No.: 44801002

Executive Summary: In a chronic toxicity/carcinogenicity study (MRID 44801002), Phosalone (94.5% a.i.) was administered in the diet to 50 Crl:CD (SD) BR rats/sex/dose [main study] for 104 weeks at dose levels of 0 ppm, 5 ppm, 50 ppm, or 1000 ppm [lowered to 500 ppm from week 27 on]. Phosalone was administered to 15 rats/sex/group (control, low-, and mid-dose levels) or 25/sex (high dose) in a satellite study at the same dose levels for 52 weeks. These dose levels corresponded to 0.2, 1.8, and 20 mg/kg/day, respectively, in males; 0.2, 2.5, 31 mg/kg/day, respectively, in females. The dosing was considered adequate, based primarily on brain cholinesterase inhibition in both sexes.

Survival was not adversely affect; in fact, both sexes at the high-dose level had the highest survival rate, and the control rats of both sexes had the lowest. The high-dose females displayed an apparent, treatment-related, increase in the incidence of hyperactivity, which dissipated following the lowering of the dose at week 27. Both sexes at the high dose displayed decreased [males 89%-92%/females 77%-84% of control] body weight throughout the first year of the study. After the high-dose level was lowered at week 27, body weight was comparable among the groups. At study termination, comparable body weights were observed among the group [high-dose males 98%/high-dose females 96% of the control]. Body-weight gains during the 0-13 week interval were decreased for both sexes at the high-dose level [males 88%/females 65% of control]. Food consumption/efficiency and water consumption were also decreased for both sexes at the high-dose level, mainly during the first year of the study. No adverse, treatment-related, effects were observed on ophthalmoscopy, hematology, clinical chemistry [except cholinesterase activity], or urinalysis in either sex. There was a dose-related inhibition of both plasma and RBC cholinesterase activities in females at all time intervals measured. In males, inhibition of both plasma and RBC cholinesterase activities was observed mainly at the high-dose level throughout the study. Brain cholinesterase activity was inhibited at the high-dose level in both sexes at study termination.

There was no apparent treatment-related increase in tumor incidence in either sex compared with concurrent controls and historical controls.

The **NOAEL** for systemic toxicity is 5 ppm **[0.2 mg/kg/day in males and females]**, and the **LOAEL** for systemic toxicity is 50 ppm **[1.8 mg/kg/day and 2.5 mg/kg/day in males and females, respectively]**, based on plasma and RBC cholinesterase inhibition [both sexes], decreased testes/epididymal weight [males], and an increased incidence of testicular lesions [tubular atrophy]. At the high-dose level, brain cholinesterase activity was decreased in both sexes [males 27% of control/females 40% of control], there was an increased incidence of hyperactivity [females], decreased adrenal weight [females], and increased incidences of pituitary hyperplasia [both sexes], vacuolated cortical cells of the adrenal [males], and aggregations of alveolar macrophages in the lungs [females]. There was no apparent increase in any tumor type that could be attributed to treatment.

This guideline chronic toxicity/carcinogenicity study is **acceptable/guideline** [OPPTS 870.4300; §83-5],

and it **satisfies** the guideline requirement for a chronic toxicity/carcinogenicity study in rats.

Dose and Endpoint for Establishing RfD: NOAEL = 0.2 mg/kg/day based on plasma and red blood cell cholinesterase inhibition in both sexes and decreased testes/epididymal weight and an increased incidence of testicular lesions (tubular atrophy) in males.

Uncertainty Factor(s): 100x (10x for interspecies extrapolation and 10x for intraspecies variabililty).

$$\textbf{Chronic RfD} = \frac{\text{0.2 mg/kg/day (LOAEL)}}{\text{100 (UF)}} = \textbf{0.002 mg/kg/day}$$

Comments about Study/Endpoint: The duration of exposure is appropriate for this endpoint. This endpoint is supported by the 1-year dog study with a threshold LOAEL of <0.17 mg/kg/day for plasma cholinesterase inhibition (<20%).

The endpoint from the 2-year rat study was selected instead of the endpoint from the 1-year dog study because the cholinesterase effects in rats were consistent in different studies and in both sexes. In contrast, plasma cholinesterase inhibition at the low-dose in the 1-year dog study was believed to be a threshold effect very near a NOAEL because the inhibition was less than 20% and occurred in only 1 sex (males). There were also confounding factors in the dog study: 3 treatment animals had surgery for inguinal hernia repair and 2 males had surgical repair of wounds from fighting which interrupted test substance administration. A dog rangefinding study was not received in time for review, but reported a NOAEL for plasma ChE inhibition of 20 ppm (0.9 mg/kg/day) and a LOAEL of 500 ppm (21.7 mg/kg/day), which lends further support for the LOAEL in the chronic dog study being a threshold effect. **This risk assessment is required.**

C. Occupational/Residential Exposure

This reregistration action was for an import tolerance. There are no registered occupational or residential uses at the present time and dermal and inhalation endpoints were not selected.

D. Margins of Exposure for Occupational/Residential Exposures

There are presently no occupational or residential uses for phosalone and MOEs for occupational or residential uses were not selected.

E. Recommendation for Aggregate (Food + Water + Residential) Risk Assessments

An aggregate assessment is not needed. There are neither residential uses for phosalone nor expected drinking water exposure because there is presently no domestic registration for phosalone.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study-Rats: OPPTS 870.4300 (OPP §83-5)

MRID No.: 44801002

Executive Summary: See the chronic RfD section of this document for the Executive Summary.

Discussion of Tumor Data: There was no evidence of treatment-related carcinogenicity.

Adequacy of the Dose Levels Tested: Doses were adequate based upon the occurrence of clinical signs, cholinesterase inhibition, organ weight changes, and microscopic pathology.

2. Carcinogenicity Study-Mice: OPPTS 870.4200 (OPP 83-2b)

Accession No: 00065653

Executive Summary: In a mouse carcinogenicity study, phosalone (95.3%) was administered for 2 years at dietary concentrations of 0, 15, 50, or 150 ppm to 65 mice (control and high-dose) or 60 mice (low- and mid-dose) per sex per dose group. Doses were equivalent to 0, 2.25, 7.5, or 22 mg/kg/day. There was an interim sacrifice of 5 mice/sex from the control and high-dose groups at 6 weeks for cholinesterase determinations. Cholinesterase activity was also determined at termination for 5 mice/sex from the control and high-dose groups.

Mortality was unaffected by treatment; survival ranged from 40-57% in the different dose groups. There was no effect of treatment upon body weight, weight gain, clinical signs, food consumption, or gross or microscopic pathology. Absolute and relative adrenal weights were increased in all treatment groups, however, this is not believed to be toxicologically significant in the absence of histological correlates.

Animals were fasted for 12 hours prior to cholinesterase determinations at 6 weeks, but not at termination. Cholinesterase activity was not determined in low- and mid-dose groups. **Brain cholinesterase** activity was unaffected by treatment. **Plasma cholinesterase** activity in the high-dose group was decreased at week 6 in males (-55%) and females (-48%) and at week 105 in males (-81%) and females (-80%) in comparison to controls. **Red blood cell cholinesterase** activity was decreased in the high-dose group at week 6 in males (-19%) and females (-29%) and at week 105 in males (-61%) and females (-24%) in comparison to controls.

Uterine leiomyomas were increased (1/60, 1/60, 2/60, 3/60) as were leiomyosarcomas (1/60, 2/60, 2/60, 4/60) in the respective dose groups. These incidences were not statistically significant and were within the historical control ranges from the performing laboratory from 1977-1981 (uterine leiomyomas ranged from 0-10% and uterine leiomyosarcomas ranged from 0-6.78%, MRID 44792011).

In this study, the **LOAEL** was 150 ppm (highest dose tested, 22 mg/kg/day) based upon inhibition of plasma and red blood cell cholinesterase activity. A **NOAEL** was not determined because cholinesterase activity was not determined in low- and mid-dose groups. The LOAEL may have been lower had cholinesterase activity been determined in low- and mid-dose groups. Dosing was judged adequate for a carcinogenicity study based upon cholinesterase inhibition, although the animals could have tolerated a larger dose. This study is classified **acceptable/guideline**.

Discussion of Tumor Data: There was no evidence of treatment-related carcinogenicity.

Adequacy of the Dose Levels Tested:. Dosing was adequate based upon inhibition of red blood cell and plasma cholinesterase activity.

3. Classification of Carcinogenic Potential:

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the HIARC classified phosalone as a **"not likely"** human carcinogen. This classification is based on the lack of evidence of carcinogenicity in male and female mice as well as in male and female rats..

IV. MUTAGENICITY

In an *in vitro* cytogenetic assay (MRID No. 41143301), Chinese hamster ovary (CHO) cells were exposed to Phosalone technical (94.35%) at concentrations of 15.0-200 μg/mL without S9 activation and 37.5-300 μg/mL with S9 activation. Cells were fixed 20 hours after treatment and examined for the frequency of structural chromosome aberrations. The S9 was derived from Aroclor 1254-induced rat livers and the test substance was delivered to the test system in dimethyl sulfoxide. Severe cytotoxicity was evident at 200 μg/mL -S9 and 300 μg/mL +S9. The positive controls induced the expected high yield of cells with aberrant chromosome morphology. There was, however, **no evidence of a clastogenic effect** induced by Phosalone technical in either the presence or absence of S9 activation. This study is classified **acceptable/guideline** and satisfies the guideline requirements for an *in vitro* mammalian cell cytogenetic assay.

In independent microbial mutagenicity assays (MRID No. 447920115, Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 250-10,000 µg/plate Phosalone technical (94%) in the initial and confirmatory trials both in the presence and absence of S9 activation. A repeat trial was also conducted with strains TA1538 and TA98 using comparable nonactivated and S9-activated doses. The S9 fraction was derived from Aroclor 1254 induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide. Compound precipitation was reported at doses $\geq$1000 µg/plate+/-S9. There was no evidence of cytotoxicity at any dose. All strains responded in the expected manner to the appropriate positive control. There was also **no indication that Phosalone technical induced a mutagenic effect at any dose with or without S9 activation**. The study is classified **acceptable/guideline** and satisfies the guideline requirements for a bacterial gene mutation assay.

In an *in vitro* unscheduled DNA synthesis (UDS) assay (MRID No. 44792016), primary rat hepatocytes

recovered from a male Fischer-344 rat were exposed to 15 doses (0.005-252 µg/mL) Phosalone technical (94%) for 18.4 hours. Recovered hepatocytes treated with 0.503-25.2 µg/mL were scored for UDS. The test material was delivered to the test system in dimethyl sulfoxide. Severe cytotoxicity was seen at >25.2 µg/mL and moderate cytotoxicity (64.7% survival) was observed at 25.2 µg/mL. The positive control induced the expected high yield of hepatocytes with net nuclear grains. There was, however, an increase in the percentage of cells in repair (18% versus 2% in the solvent control) at 25.5 µg/mL; the increase in net nuclear grain counts at this dose (2.1 versus -1.09 in the solvent control) did not exceed the criterion used to indicate UDS activity. The response was limited to this dose with no evidence of UDS at lower levels (0.503-10.1 µg/mL). Based on these findings, Phosalone technical is considered to be **weakly active for UDS induction** in this *in vitro* test system. **It is recommended that the assay be repeated to confirm or refute the findings of this study.** The study is classified **acceptable/guideline** and satisfies the requirements for FIFRA Test Guideline 84-2 for a UDS assay.

V. FQPA CONSIDERATIONS

1. Adequacy of database:

NOAELs were not determined for plasma ChE inhibition in the acute rat neurotoxicity study; for systemic effects or plasma, RBC, or brain ChE inhibition in the subchronic rat neurotoxicity study; for plasma ChE inhibition in the chronic dog study; for plasma or RBC ChE inhibition in the mouse carcinogenicity study; or for RBC ChE inhibition in the reproduction study. The lack of NOAELs in these studies did not interfere with endpoint selection and the toxicology database is considered adequate and of good quality. The HIARC recommended that the unscheduled DNA synthesis assay be repeated to confirm or refute results in the submitted study. No relevant developmental or reproductive studies were found following a literature search.

2. Neurotoxicity:

(a) In an acute delayed neurotoxicity study (Accession 00252078), 20 New Hampshire Red Hens received an oral dose of 600 mg/kg technical phosalone (% a.i. not reported) in a dosing volume of 4 mL/kg corn oil on days 0 and 22. A negative control group of 10 hens received corn oil on days 0 and 22. A positive control group of 10 hens received a single oral dose of 750 mg/kg TOCP in corn oil on day 0 and second positive control group of 10 hens was similarly treated on day 22. Hens in the phosalone and positive control groups received i.m. injections of 3 mL/kg atropine 15 minutes before treatment. An acute oral toxicity study (MRID 00137038) had previously determined an oral LD50 of 503 mg/kg phosalone in hens.

Hens were observed twice daily for clinical signs and neurotoxicity. Hens in the phosalone treatment group and in the negative control group were sacrificed 3 weeks after the second treatment. Hens in the positive control groups were sacrificed 3 weeks after their respective treatments. Hens were perfused with 4% formaldehyde and 1% glutaraldehyde for histopathological examination of spinal cord and sciatic and tibial nerves. Neurotoxic esterase and cholinesterase activity were not determined.

In the positive control groups, 3/20 birds were sacrificed moribund; histopathological lesions and clinical

signs of delayed neurotoxicity were observed. In the phosalone treatment group there were 2/20 acute deaths from the first dose and 7/18 deaths from the second dose. Clinical signs in the phosalone treatment group included excitement, prostration, and ataxia on day 2; there were no histopathological lesions or clinical signs of delayed neurotoxicity in this group. This study was **negative** for delayed neurotoxicity in hens after treatment with phosalone. Although the % a.i. was not reported and cholinesterase and neurotoxic esterase determinations were not made, this study is classified **acceptable/guideline** and **satisfies** requirements for a delayed neurotoxicity study in hens.

(b) The executive summary for the acute neurotoxicity study is reported in the acute RfD section of this document.

(c) In a subchronic neurotoxicity study (MRID 44852504), 10 Crl:CD BR rats/sex/dose group received dietary concentrations of 0, 50, 150, or 600 ppm phosalone (93.8%) in the diet for 13 or 14 weeks. Dietary concentrations were equivalent to 0, 3.9, 11.5, or 45.9 mg/kg/day for males and 0, 4.4, 12.6, or 56.0 mg/kg/day for females. Rats were given functional observational battery and motor activity tests pre-dose, and at 4, 8, and 13 weeks. After 13 weeks of treatment, rat were sacrificed and 5 rats/sex/dose group were perfused for microscopic examination of the nervous system and 5 rats/sex/dose group had plasma, red blood cell (RBC), and brain cholinesterase (ChE) activity tested by the Ellman method. An additional 10 rats/sex/group comprised a satellite group from which 5 rats/sex/dose were sacrificed at 4 and 8 weeks for brain ChE measurements. RBC and plasma ChE were also determined in this group at pretest and at weeks 4 and 8.

No deaths or clinical signs occurred in the study. Females in the high-dose group had reduced body weights (-10% to -14% of controls) throughout the study. Body weight gain in high-dose females after 13 weeks of treatment was reduced in comparison to controls (-28%). Body weights and weight gains in other groups were comparable to controls. Food consumption and food efficiency were generally comparable among the different groups. Forelimb and hindlimb grip strength were decreased in high-dose males and/or females at various time intervals. Landing footsplay values were decreased in high-dose males and in all female treatment groups in several time intervals. Also reported were increased incidence of hairloss and "badly groomed" in high-dose females at week 13.

Plasma cholinesterase (ChE) was inhibited in mid- and high-dose males and in all female treatment groups. Red blood cell ChE was inhibited in mid-dose and high-dose males and in all female treatment groups. Brain ChE was inhibited in all male treatment groups and in mid- and high-dose females.

The **LOAEL** is 50 ppm, the lowest dose tested **(males: 3.9 mg/kg/day; females: 4.4 mg/kg/day)**, based upon inhibition of brain ChE activity in males and inhibition of plasma and RBC ChE activity in females; a **NOAEL** was not established. This study is classified **acceptable/guideline**.

(d) In the time-of-peak-effect study (MRID 44852501), 10 rats/sex were subjected to a single exposure (by gavage) to Phosalone Technical at a dose of 60 mg/kg to determine the time of peak effect. The animals were observed for evidence of signs of toxicity 4, 5, 6, 7, and 8 hours post-dosing and daily thereafter until the termination of the study (Day 8). Numerous signs of toxicity (excessive salivation, tremors, hunched posture, unsteady gait, hypothermia, piloerection, wet urogenital region and labored breathing) were evident shortly after dosing with a preponderance of the effects being observed at the 6

and 7 hour observation period. Although males had completely recovered by Day 2 of the study, signs of toxicity were still apparent in females. Plasma and erythrocyte cholinesterase activity (PChE and RChE) were measured prior to dosing, and at 2.5, 4, 6, 8, and 24 hours after administration of the test substance while brain cholinesterase activity was measured on day 8 of the study. Statistically significant ($p \leq 0.01$) PChE inhibition (both sexes) was first seen 2.5 hours after exposure to the test article and persisted until the 24 hour measurement period. RChE inhibition was first reported at the 4 hour observation period, however, the pattern of inhibition over observation time points was not as consistent as that of PChE. Measurements of brain cholinesterase activity at the end of the study period (Day 8) revealed a statistically significant level of BChE inhibition for both males and females. Given the extent of clinical signs seen at different time points, the time-of-peak-effect was established to be 6 hours after exposure to the test article.

3. Developmental Toxicity

(a) Rat developmental toxicity study: In a developmental toxicity study, 25 Wistar/HAN rats/dose group were given nominal doses of 0, 2, 10, or 20 mg/kg/day phosalone (93.6%) in 4% carboxymethylcellulose from gestation days 6-15. Corrected for analytical concentrations, doses were 0, 1.7, 8.6, or 16.6 mg/kg/day. Dams were euthanized on day 21 and uteri, ovaries, and fetuses examined. Cholinesterase measurements were not made.

No deaths occurred in the study. Clinical signs were limited to the high-dose group and continued during the post-treatment period. All animals in this group experienced continuous chewing motions, hypersensitivity to noise, piloerection, and dyspnea. Body weights were similar in all dose groups. Body weight gain in the high-dose group was decreased in comparison to controls during the treatment period (-34%) as was food consumption (-10%).

Pregnancy rates and mean numbers of corporae lutea and implants were similar among groups. There was an increased number of resorptions in the high-dose group (32 vs 17 in controls) with a corresponding increase in post-implantation loss (10% vs 5.1% in controls) and a slight decrease in the number of live fetuses/dam (12.0 vs 12.7 in controls).

The **NOAEL** for **maternal** toxicity is 8.6 mg/kg/day and the maternal LOAEL is 16.6 mg/kg/day based upon clinical signs and decreased body weight gain. The **NOAEL** for **developmental** toxicity is 8.6 mg/kg/day and the LOAEL is 16.6 mg/kg/day based on increased resorptions and post-implantation loss. This study is classified **acceptable/guideline** and satisfies requirements for developmental toxicity study in rats.

(b) Rabbit developmental toxicity study: See the Acute RfD for females 13+ section in this document for the executive summary.

4. Reproductive Toxicity:

In a 2-generation reproduction study [MRID 44792013], Phosalone [94.5% a.i.] was administered to 32 [F0]/28 [F1] Crl:CD(SD)VAF/Plus BR rats/sex/dose via the diet at dose levels of 0, 10, 50, and 400 ppm [F0 males 0.71, 3.56, 29.39 /females 0.78, 3.94, 32.82 mg/kg/day; F1 males 0.8, 3.98, 33.58/females 0.86, 4.3, 36.74 mg/kg/day, respectively] during the pre-mating period of 10 weeks [F0 generation]/16

weeks [F1 generation]. There was one litter per generation.

There were no treatment-related deaths of the parental rats in either generation. There were no adverse, treatment-related, effects on body weight, body-weight gain, or food consumption in either sex or generation during the dosing period, although at the high-dose level, both sexes of the F1 offspring displayed a significantly lower body weight [89% of control] at the start of dosing at week 4. A slight decrease in water consumption was observed for both sexes, mainly at the high-dose level, in both generations. For the dams, although comparable body weights were observed among the F0-generation dams throughout gestation and lactation, body-weight gains were decreased throughout gestation [87%-93% of control] and lactation [69%-70% of control] at the high-dose level. For the F1-generation dams, slightly lower body weights [93%-95% of control] were observed at the high-dose level, mainly during lactation, and body-weight gains were decreased [80%-82% of control] also, mainly during lactation.

Pregnancy rates were comparable among the groups in both generations, and the majority of the females mated within the first four days after pairing. In both generations, there were no significant differences among the groups with respect to the duration of gestation, the number of implantation sites, implantation losses [%], or sex ratios, and the majority of the dams delivered live fetuses.

There was a dose-related decrease in plasma and erythrocyte cholinesterase activity in both sexes and in both generations at study termination, and the magnitude of the decreases was similar for both generations. The decrease in RBC cholinesterase activity occurred at all dose levels in the F0 males and the F1 females. Although brain tissue samples were collected for brain cholinesterase activity measurements, the analyses were not performed. There were no apparent, treatment-related, effects on organ weights, and gross and microscopic findings were comparable among the groups for both sexes and both generations.

There was an increase in pup death between birth and day 4 *post partum* of the offspring of both generations, although the effect was greater in the first generation. At birth, body weights of the F1 offspring were comparable among the groups, but by day 4 *post partum*, body weight was significantly reduced [91% of control] at the high-dose level compared to the control and remained lower throughout lactation [90% of control]. Slightly lower body weights [95% of control] were observed at birth of the F2 pups at the high-dose level, and decreased body weights [84%-87% of control] were observed throughout lactation at the high-dose level, with the magnitude of the deficit increasing with time. Similarly, litter weights were comparable at birth in both generations, but by day 4 and throughout lactation, decreased litter weights were observed at the high-dose level in both generations. There was a marginal delay at the high-dose level in surface righting and startle response in the F1 weanlings and a marginal delay in male post-weaning development [balanopreputial cleavage]. There were no apparent adverse effects observed on pups at either the mid- or low-dose level.

No **NOAEL for maternal/paternal toxicity** was determined, based on decreased erythrocyte [RBC] cholinesterase activity at all dose levels in F0 males and F1 females at 10 ppm [F0 males 0.7/F0 females 0.8 mg/kg/day; F1 males 0.8/F1 females 0.9 mg/kg/day], which is the **LOAEL**. Decreased plasma and/or RBC cholinesterase activities in both sexes was observed at 50 ppm [F0 males 3.6/F0 females 3.9 mg/kg/day; F1 males 4.0/F1 females 4.3 mg/kg/day]. The **reproductive NOAEL** is 400 ppm [F0 males 29.4/F0 females 32.8 mg/kg/day; F1 males 33.6/F1 females 36.7 mg/kg/day], the highest dose tested. The **neonatal NOAEL** is 50 ppm [F0 males 3.6/F0 females 3.9 mg/kg/day; F1 males 4.0/F1 females 4.3

mg/kg/day], and the **LOAEL** is 400 ppm [F0 males 29.4/F0 females 32.8 mg/kg/day; F1 males 33.6/F1 females 36.7 mg/kg/day], based on increased mortality during the day 0-4 *post partum* interval in both generations, and decreased mean pup body weight and litter weight.

This guideline [§83-4; OPPTS 870.3800] 2-generation reproduction study in rats is classified **acceptable/guideline**.

5. Determination of Susceptibility:

The HIARC evaluated the rat and rabbit developmental toxicity studies and the 2-generation reproduction study with phosalone to determine whether there was evidence of enhanced susceptibility of offspring. No literature studies were available which reported adverse developmental or reproductive effects with phosalone.

In the rat developmental toxicity study, the maternal LOAEL of 16.6 mg/kg/day was based upon clinical signs (labored breathing, hypersensitivity to noise, continuous chewing, piloerection) and the developmental LOAEL of 16.6 mg/kg/day was based on increased resorptions. The maternal and developmental NOAELs were both 8.6 mg/kg/day indicating no quantitative increase in fetal sensitivity. The maternal LOAEL for this study is likely to be an underestimate of maternal toxicity since cholinesterase activity was not determined and data from other studies indicate that cholinesterase inhibition would have occurred at much lower levels. The increase in resorptions was not considered indicative of qualitative susceptibility since this effect occurred only at the high dose in the presence of severe maternal toxicity.

In the rabbit developmental toxicity study, the developmental LOAEL of 10 mg/kg/day was based upon post-implantation loss and the maternal LOAEL of 20 mg/kg/day was based upon clinical signs of toxicity and statistically significant decreases in food consumption. The developmental NOAEL (1 mg/kg/day) was lower than the maternal NOAEL (10 mg/kg/day) indicating a quantitative increase in fetal sensitivity in rabbits following *in utero* exposures to phosalone. There were two reasons for considering that this was not a true quantitative increase in fetal sensitivity.

First, the selected endpoint is a very conservative indicator of toxicity because it is based on total resorptions and is not a litter effect. Mean resorptions were increased but not with statistical significance. Total resorptions achieved statistical significance and were primarily attributed to 1 dam with total resorptions in the mid-dose group and 2 dams with total resorptions in the high-dose group.

Secondly, cholinesterase activity was not determined in this study. Considering the severity of the maternal clinical signs (labored breathing, abdominal cramps, extension spasms, prostration) occurring at 20 mg/kg/day, it is likely that significant cholinesterase inhibition was occurring at this dose. Based upon information from other studies (e.g. the acute neurotoxicity study in rats in which plasma cholinesterase inhibition was observed after a single oral dose of 10 mg/kg and the 2-generation study in which repeated dietary doses of 0.086 mg/kg/day resulted in red blood cell cholinesterase inhibition), it is presumed that cholinesterase activity could have been inhibited in the maternal rabbits at 10 mg/kg/day which could have caused the fetal effects. Therefore, it was considered unlikely that there was a true quantitative increase in fetal sensitivity.

In the two-generation reproduction study in rats, the parental LOAEL was <0.86 mg/kg/day, the lowest

dose tested, and was based on red blood cell cholinesterase inhibition. Clinical signs in parents were not reported at the high dose, however, parental red blood cell cholinesterase inhibition was significant (-74% of controls). The neonatal LOAEL was 29.39 mg/kg/day, the highest dose tested and was based upon pup mortality between days 1-4. The mortality in pups was not considered indicative of qualitative susceptibility since this effect occurred only at the high dose in the presence of severe parental toxicity.

6. Hazard-based Recommendation of the FQPA Safety Factor:

The application of an FQPA factor to ensure the protection of infants and children from exposure to phosalone, as required by FQPA, will be determined by the FQPA Safety Factor Assessment Review Committee. The HIARC recommended a 1x safety factor for the protection of infants and children under FQPA since unequivocal evidence of neither qualitative nor quantitative susceptibility was identified (see discussion above).

7. Recommendation for a Developmental Neurotoxicity Study

Based on the following weight-of-evidence considerations, the HIARC did not recommend a developmental neurotoxicity study in rats for phosalone.

(i) Evidence that suggests requiring a developmental neurotoxicity study: Phosalone causes cholinergic signs and plasma, red blood cell, and brain cholinesterase inhibition.

(ii) Evidence not supporting a developmental neurotoxicity study: No changes in brain weight, brain dimensions, or nervous system histopathology were noted in acute and chronic neurotoxicity studies, nor in chronic dog, rat, or mouse studies. No alterations in development of the central nervous system were observed in the rat and rabbit developmental studies. No observations indicative of neurotoxicity were reported in offspring in the 2-generation reproduction study. Phosalone was negative for delayed neuropathy in a hen study.

VI. HAZARD CHARACTERIZATION

Phosalone is an organophosphate insecticide with a toxic mode-of-action by way of inhibition of cholinesterase (ChE) enzyme. The acute oral toxicity category is II. There was no consistent sensitivity of either sex in various studies. With the exception of the chronic dog study, in which the systemic no observed adverse effect level (NOAEL) was based on decreased body weight gain, all endpoints in adult animals were based on either ChE inhibition or clinical signs related to ChE inhibition. Plasma and red blood cell ChE inhibition had similar NOAELs in several studies. NOAELs were not established in several studies (see FQPA Considerations, Adequacy of the Database, in this document). Offspring effects were based upon increased resorptions in the rat and rabbit developmental studies and upon increased pup mortality in 2-generation reproduction study.

The carcinogenic classification is "not likely" to be a human carcinogen based upon results in the combined chronic toxicity/carcinogenicity study in rats and the carcinogenicity study in mice. An *in vitro* cytogenetics assay was negative for clastogenic effects. An Ames assay was also negative. An *in vitro* unscheduled DNA synthesis assay was weakly active and it was recommended that this assay be repeated to confirm questionable results. Conclusions of the HIARC are not expected to change based on results

from this study. The database for phosalone is otherwise complete and of good quality.

VIII. RECOMMENDATION FOR ADDITIONAL STUDIES

The database is complete with the exception of a required unscheduled DNA synthesis (UDS) assay. The UDS assay is required to confirm questionable results in the submitted UDS assay.

IX. ACUTE TOXICITY

The acute toxicity of phosalone as reported in the 1987 Toxicology Chapter is summarized below in Table 1.

Table 1. Acute Toxicity Profile of Phosalone.

GDLN	Study Type	MRID	Results	Tox Category
81-1	Acute Oral	00006716, 00006643	Male: 120-155 mg/kg Female: 90-135 mg/kg	II
81-2	Acute Dermal, Rat	00006643	Female: 390 mg/kg	III
81-2	Acute Dermal, Rabbit	00006643	> 1000 mg/kg	III
81-7	Delayed Neurotoxicity, Hen	00137037 00137038	Negative for OPIDN	—

X. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

TABLE 2. Toxicological endpoints for use in human risk assessment

EXPOSURE	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (General population including infants and children)	LOAEL = 10	Plasma ChE inhibition	Acute neurotoxicity in rats
	UF =300	**Acute RfD = 0.03 mg/kg /day**	
Acute Dietary (Females 13+)	Developmental NOAEL = 1	Post-implantation loss	Developmental toxicity in rabbits
	UF =100	**Acute RfD= 0.01 mg/kg /day**	
Chronic Dietary	NOAEL = 0.2	Plasma and RBC ChE inhibition (both sexes), decreased testicular weight and lesions	2-Year Rat Study
	UF =100	**Chronic RfD = 0.002 mg/kg/day**	
Dermal or Inhalation Endpoints	---	None selected. The phosalone reregistration action is for an import tolerance.	—